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United States Patent [19][11] **Patent Number:** **5,985,932****MacLean et al.**[45] **Date of Patent:** **Nov. 16, 1999**[54] **INHIBITION OF AUTOIMMUNE DISEASES**[75] Inventors: **David B. MacLean**, Providence, R.I.;
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Conn.[73] Assignee: **Pfizer Inc**, New York, N.Y.[21] Appl. No.: **08/804,346**[22] Filed: **Feb. 21, 1997****Related U.S. Application Data**

[60] Provisional application No. 60/012,402, Feb. 28, 1996.

[51] **Int. Cl.⁶** **A61K 31/135**[52] **U.S. Cl.** **514/648**[58] **Field of Search** 514/648[56] **References Cited**

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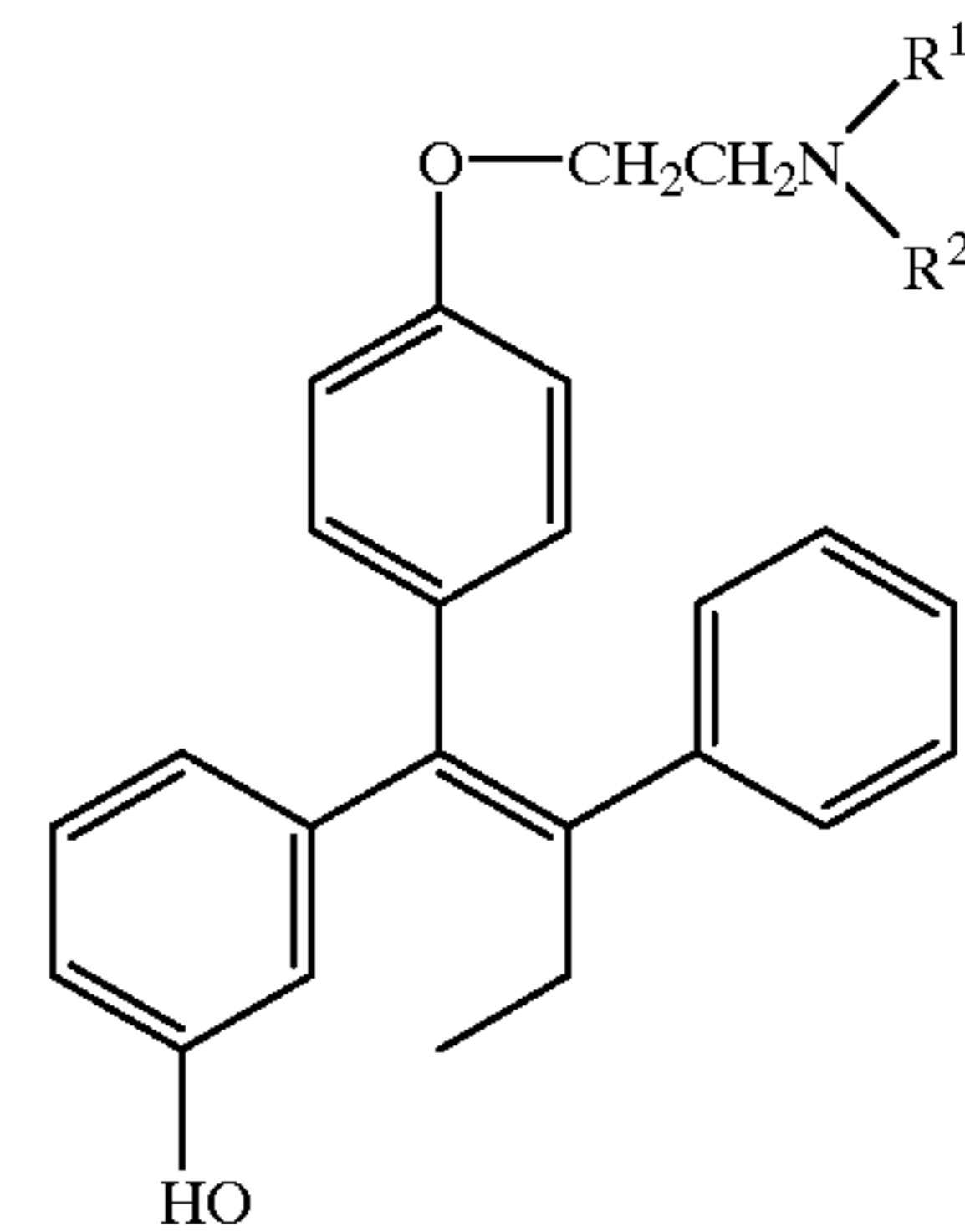
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The present invention provides novel methods of inhibition of autoimmune diseases comprising administering to a mammal in need of treatment an effective amount of a compound of formula I



wherein

R¹ and R² may be the same or different provided that, when R¹ and R² are the same, each is a methyl or ethyl group, and, when R¹ and R² are different, one of them is a methyl or ethyl group and the other is hydrogen or a benzyl group; or a pharmaceutically acceptable salt thereof.**3 Claims, No Drawings**

INHIBITION OF AUTOIMMUNE DISEASES

This is a continuation of provisional application 60/012, 402 filed Feb. 28, 1996, the benefit of which is hereby claimed under 37 C.F.R. §1.78(a)(3).

BACKGROUND OF INVENTION

Autoimmune diseases involve aberrant regulation of cellular and humoral mediated immunity and are frequently associated with abnormal or enhanced T cell, B cell and macrophage effector functions directed towards self antigens. The activation of these cellular components towards self antigens is believed related to the break in feedback mechanisms associated with self tolerance. Autoimmune diseases encompass a whole spectrum of clinical entities and despite the differences in the target organ have many similarities. These include their preponderance in females of child bearing age with a female to male ratio varying from 50:1 in Hashimoto's thyroiditis to 10:1 in Systemic lupus erythematosus (SLE) to 2:1 in Myasthenia gravis (Ahmed et al., *Am J. Path.*, 121:531 (1985)). In addition, these diseases are all characterized by their chronicity, the tendency of clinical remission and "flare ups" for poorly understood reasons, and the involvement of other organs. While the presence of autoantibodies, inappropriate expression of class II antigens, macrophage activation and T cell infiltration to the target organ have been described in essentially all of the autoimmune diseases, neither the triggering mechanisms which result in disease activation nor disease progression are well understood. Accordingly, therapy for these diseases is largely unsatisfactory and involves the use of gold salts, methotrexate, antimalarials, glucocorticoids (methylprednisolone), and other immunosuppressives as well as plasmapheresis and attempts at inducing tolerance. Treatment of autoimmune diseases has not improved significantly over the past decade and primarily is associated with the use of nonsteroidal and steroidal anti-inflammatory agents to treat the symptoms of the disease. Clearly while suppression of the specific immune response directed against the host is necessary, generalized immunosuppression as with glucocorticoids has major liabilities in terms of side effect profile and the propensity of the immunosuppressed patient to be at greater risk for other infectious and non-infectious diseases.

Estrogen appears to be involved with autoimmune diseases although its role in disease progression or regression is complex and dependent on the nature of the autoimmune disease. Estrogen for example appears to have an ameliorating effect on rheumatoid arthritis while having an exacerbating effect on systemic lupus (Chander & Spector; *Ann. Rheum. Dis.* 50:139). Estrogen has been demonstrated to have a suppressive role on T cell function and yet an immunostimulatory effect on B cells. Therefore, estrogen-like compounds should prove beneficial in diseases associated with activated T cells including rheumatoid arthritis, multiple sclerosis, Guillan Barre syndrome and Hashimoto's thyroiditis through inhibition of T cell function (Holmadahl, *J. Autoimmun.* 2:651 (1989)).

In addition to the suppressive effects of estrogen on T cells, estrogen may have additional protective roles. Marui et al., (*J. Clin. Invest.* 92:1866 (1993)) have recently reported that antioxidants suppress endothelial expression of VCAM-1. VCAM-1 is the ligand for VLA4, the T cell and macrophage integrin associated with trafficking of these cells out of the vasculature and into the perivascular space and target organs. As estrogen is an antioxidant, it would be

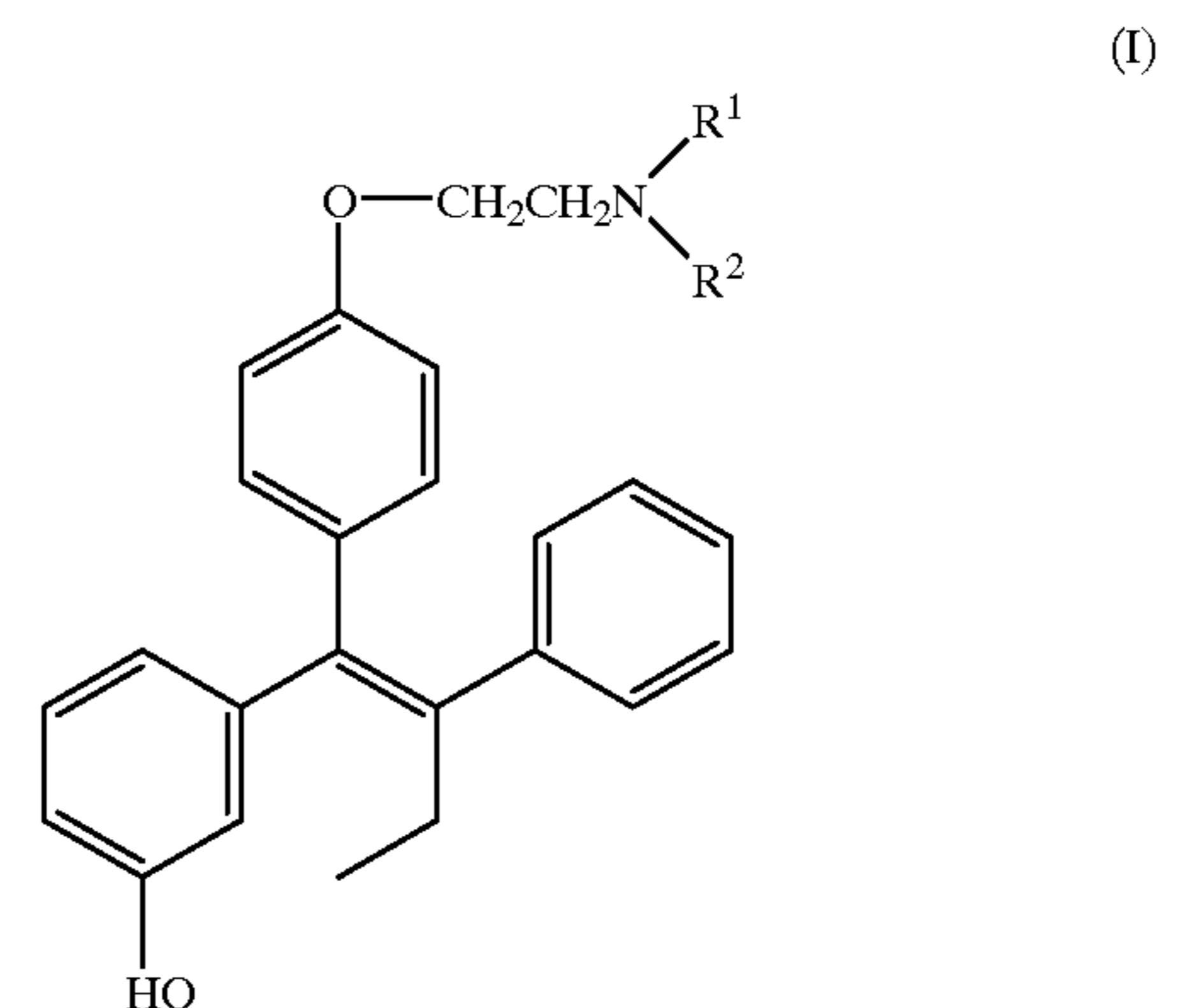
anticipated that estrogen and related analogs will inhibit VLA4 dependent trafficking of cells and thus hinder the immune cascade associated with autoimmune mediated disease.

Estrogen plays a detrimental role in other autoimmune diseases including systemic lupus and glomerulonephritis, diseases associated with immune complexes. While the mechanism(s) responsible for estrogen mediated disease progression are not known, the ability of estrogen to increase Fc mediated phagocytosis (Friedman et al., *J. Clin. Invest.* 75:162 (1985)), and class II antigen expression and IL-1 production by macrophages from estrogen treated rodents (Flynn, *Life Sci.*, 38:2455 (1986)) has been reported. Enhancement of these macrophage mediated effector functions would be expected to contribute towards the immune cascade associated with self destruction.

It is reported in EP 664123 A1 that certain 2-phenyl-3-aryloylbenzothiophenes are effective inhibitors of autoimmune diseases.

SUMMARY OF THE INVENTION

The present invention relates to methods for inhibition of autoimmune diseases comprising administering to a mammal in need of treatment an effective amount of a compound of formula I



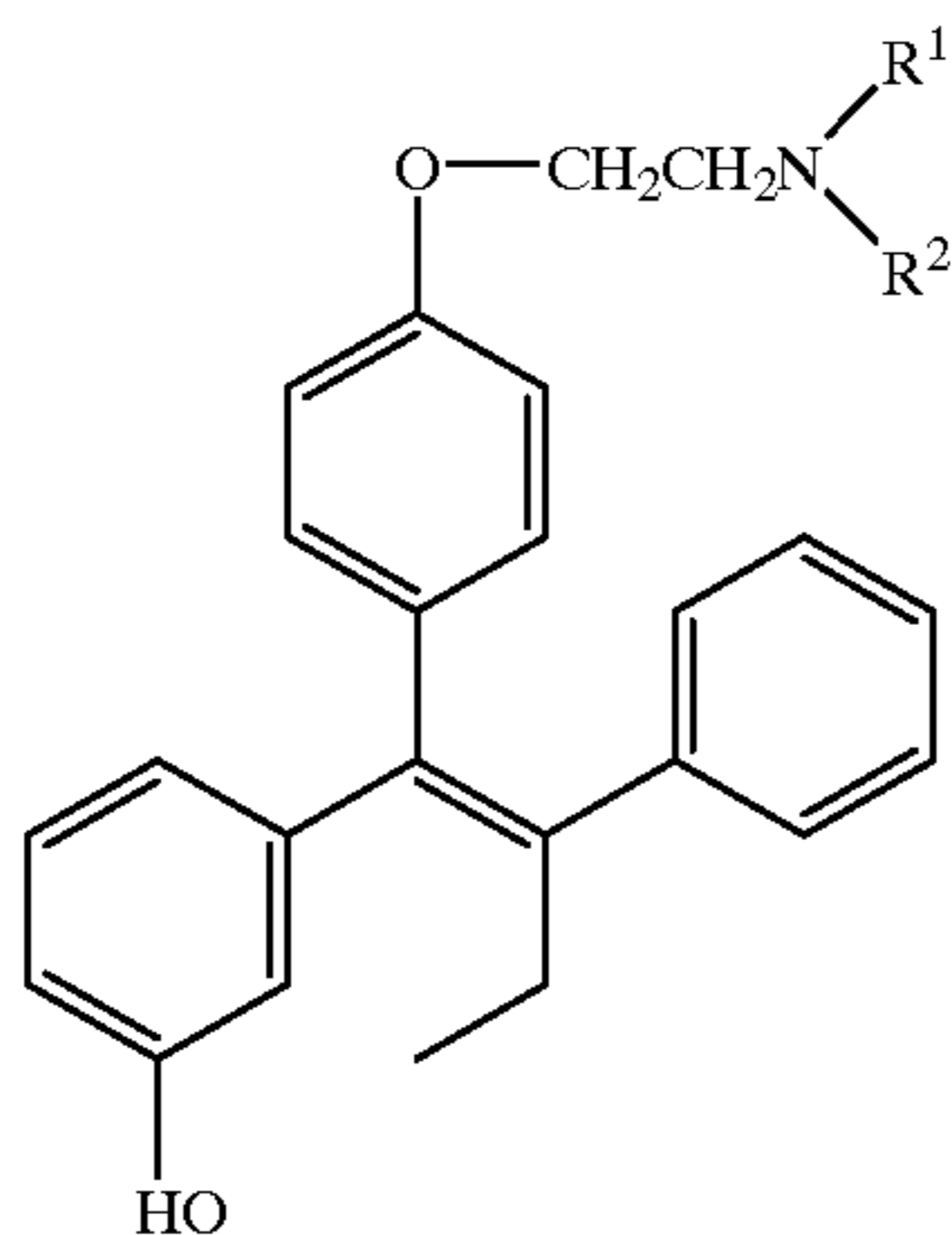
wherein R^1 and R^2 may be the same or different provided that, when R^1 and R^2 are the same, each is a methyl or ethyl group, and, when R^1 and R^2 are different, one of them is a methyl or ethyl group and the other is hydrogen or a benzyl group; or a pharmaceutically acceptable salt thereof. A preferred compound of formula I is that in which R^1 and R^2 are methyl. A preferred salt is the citrate salt.

DETAILED DESCRIPTION OF THE INVENTION

The present invention concerns methods for inhibition of autoimmune diseases. The term "inhibit" is defined to include its generally accepted meaning which includes prophylactically treating a subject to prevent the occurrence of one or more of these disease states, holding in check the symptoms of such a disease state, and/or treating such symptoms. Thus, the present methods include both medical therapeutic and/or prophylactic treatment, as appropriate.

The methods of this invention are practiced by administering to a mammal in need of treatment an effective amount of a compound formula I

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wherein R^1 and R^2 may be the same or different provided that, when R^1 and R^2 are the same, each is a methyl or ethyl group, and, when R^1 and R^2 are different, one of them is a methyl or ethyl group and the other is hydrogen or a benzyl group; or a pharmaceutically acceptable salt thereof.

Compounds of formula I are known in the art and essentially are prepared via the methods described in U.S. Pat. No. 5,047,431, which is hereby incorporated herein by reference.

A preferred formula I compound is that in which R^1 and R^2 each are methyl. This preferred compound is known as droloxifene, (E)-1-[4'-(2-Dimethylaminoethoxy)phenyl]-1-(3-hydroxyphenyl)-2-phenylbut-1-ene, which previously has been described as an antiestrogenic agent and is useful for the treatment of hormone dependent mammary tumors (U.S. Pat. No. 5,047,431), and for the relief of bone diseases caused by the deficiency of estrogen or the like (U.S. Pat. No. 5,254,594). Furthermore, droloxifene is known to have less uterotrophic effect than other antiestrogenic compounds such as tamoxifen.

Although the free-base form of formula I compounds can be used in the methods of the present invention, it is preferred to prepare and use a pharmaceutically acceptable salt form. Thus, the compounds used in the methods of this invention form pharmaceutically acceptable acid and base addition salts with a wide variety of inorganic and, preferably, organic acids and include the physiologically acceptable salts which are often used in pharmaceutical chemistry. Such salts are also part of this invention. Typical inorganic acids used to form such salts include hydrochloric, hydrobromic, hydroiodic, nitric, sulfuric, phosphoric, hypophosphoric, and the like. Salts derived from organic acids, such as aliphatic mono and dicarboxylic acids, phenyl substituted alkanolic acids, hydroxyalkanoic and hydroxyalkandioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, may also be used. Such pharmaceutically acceptable salts thus include acetate, phenylacetate, trifluoroacetate, acrylate, ascorbate, benzoate, chlorobenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, methylbenzoate, o-acetoxybenzoate, naphthalene-2-benzoate, bromide, isobutyrate, phenylbutyrate, β -hydroxybutyrate, butyne-1,4-dioate, hexyne-1,4-dioate, caprate, caprylate, chloride, cinnamate, citrate, formate, fumarate, glycollate, heptanoate, hippurate, lactate, malate, maleate, hydroxymaleate, malonate, mandelate, mesylate, nicotinate, isonicotinate, nitrate, oxalate, phthalate, terephthalate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, propiolate, propionate, phenylpropionate, salicylate, sebacate, succinate, suberate, sulfate, bisulfate, pyrosulfate, sulfite, bisulfite, sulfonate, benzenesulfonate, p-bromophenylsulfonate,

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chlorobenzenesulfonate, ethanesulfonate, 2-hydroxyethanesulfonate, methanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, p-toluenesulfonate, xylenesulfonate, tartarate, and the like. A preferred salt is the citrate salt.

The pharmaceutically acceptable acid addition salts are typically formed by reacting a compound of formula I with an equimolar or excess amount of acid. The reactants are generally combined in a mutual solvent such as diethyl ether or benzene. The salt normally precipitates out of solution within about one hour to 10 days and can be isolated by filtration or the solvent can be stripped off by conventional means.

The pharmaceutically acceptable salts of formula I compounds generally have enhanced solubility characteristics compared to the compound from which they are derived, and thus are often more amenable to formulation as liquids or emulsions.

Once prepared, the free base or salt form of formula I compounds can be administered to an individual in need of treatment for the methods herein described. The following nonlimiting test examples illustrate the methods of the present invention.

For the methods of the present invention, compounds of Formula I are administered continuously, or from 1 to 4 times daily.

As used herein, the term "effective amount" means an amount of compound of the methods of the present invention which is capable of inhibiting the symptoms of the pathological conditions herein described. The specific dose of a compound administered according to this invention will, of course, be determined by the particular circumstances surrounding the case including, for example, the compound administered, the route of administration, the state of being of the patient, and the severity of the pathological condition being treated. A typical daily dose will contain a nontoxic dosage level of from about 0.25 mg to about 100 mg/day of a compound of the present invention. Preferred daily doses generally will be from about 1 mg to about 40 mg/day.

The compounds of this invention can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. These compounds preferably are formulated prior to administration, the selection of which will be decided by the attending physician. Typically, a formula I compound, or a pharmaceutically acceptable salt thereof, is combined with a pharmaceutically acceptable carrier, diluent or excipient to form a pharmaceutical formulation.

The total active ingredients in such formulations comprises from 0.1% to 99.9% by weight of the formulation. By "pharmaceutically acceptable" it is meant the carrier, diluent, excipients, and/or salt must be compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof.

Pharmaceutical formulations containing a compound of formula I can be prepared by procedures known in the art using well known and readily available ingredients. For example, the compounds of formula I can be formulated with common excipients, diluents, or carriers, and formed into tablets, capsules, suspensions, powders, and the like. Examples of excipients, diluents, and carriers that are suitable for such formulations include the following fillers and extenders such as starch, sugars, mannitol, and silicic derivatives binding agents such as carboxymethyl cellulose and other cellulose derivatives, alginates, gelatin, and polyvinyl-pyrrolidone; moisturizing agents such as glycerol;

disintegrating agents such as calcium carbonate and sodium bicarbonate agents for retarding dissolution such as paraffin resorption accelerators such as quaternary ammonium compounds; surface active agents such as cetyl alcohol, glycerol monostearate; adsorptive carriers such as kaolin and bentonite; and lubricants such as talc, calcium and magnesium stearate, and solid polyethyl glycols.

The compounds also can be formulated as elixirs or solutions for convenient oral administration or as solutions appropriate for parenteral administration, for example, by intramuscular, subcutaneous or intravenous routes.

Additionally, the compounds are well suited to formulation as sustained release dosage forms and the like. The formulations can be so constituted that they release the active ingredient only or preferably in a particular physiological location, possibly over a period of time. The coatings, envelopes, and protective matrices may be made, for example, from polymeric substances or waxes.

Compounds of formula I generally will be administered in a convenient formulation. The following formulation examples only are illustrative and are not intended to limit the scope of the present invention.

In the formulations which follow, "active ingredient" means a compound of formula I, or a salt thereof.

Formulation 1: Gelatin Capsules
Hard gelatin capsules are prepared using the following:

Ingredient	Quantity (mg/capsule)
Active ingredient	0.25–100
Starch, NF	0–650
Starch flowable powder	0–50
Silicone fluid 350 centistokes	0–15

A tablet formulation is prepared using the ingredients below:
Formulation 2: Tablets

Ingredient	Quantity (mg/tablet)
Active ingredient	0.25–100
Cellulose, microcrystalline	200–650
Silicon dioxide, fumed	10–650
Stearate acid	5–15

The components are blended and compressed to form tablets.

Alternatively, tablets each containing 0.25–100 mg of active ingredient are made up as follows:
Formulation 3: Tablets

Ingredient	Quantity (mg/tablet)
Active ingredient	0.25–100
Starch	45
Cellulose, microcrystalline	35
Polyvinylpyrrolidone (as 10% solution in water)	4
Sodium carboxymethyl cellulose	4.5
Magnesium stearate	0.5
Talc	1

The active ingredient, starch, and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°–60° C. and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 60 U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets.

Suspensions each containing 0.25–100 mg of medicament per 5 ml dose are made as follows:

Formulation 4: Suspensions

Ingredient	Quantity (mg/5 ml)
Active ingredient	0.25–100 mg
Sodium carboxymethyl cellulose	50 mg
Syrup	1.25 mg
Benzoic acid solution	0.10 mL
Flavor	q.v.
Color	q.v.
Purified Water to	5 mL

The medicament is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form smooth paste. The benzoic acid solution, flavor, and color are diluted with some of the water and added, with stirring. Sufficient water is then added to produce the required volume. An aerosol solution is prepared containing the following ingredients:

Formulation 5: Aerosol

Ingredient	Quantity (% by weight)
Active ingredient	0.25
Ethanol	25.75
Propellant 22 (Chlorodifluoromethane)	70.00

The active ingredient is mixed with ethanol and the mixture added to a portion of the propellant 22, cooled to 30° C., and transferred to a filling device. The required amount is then fed to a stainless steel container and diluted with the remaining propellant. The valve units are then fitted to the container.

Suppositories are prepared as follows:

Formulation 6: Suppositories

Ingredient	Quantity (mg/suppository)
Active ingredient	250
Saturated fatty acid glycerides	2,000

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimal necessary heat. The mixture is then poured into a suppository mold of nominal 2 g capacity and allowed to cool.

An intravenous formulation is prepared as follows:

Formulation 7: Intravenous Solution

Ingredient	Quantity
Active ingredient	20 mg
Isotonic saline	1,000 mL

The solution of the above ingredients is intravenously administered to a patient at a rate of about 1 mL per minute.

ASSAYS

Assay 1

The procedure as set out in Holmdahl et al., Clin. Exp. Immunol., 70, 373–378 (1987) (herein incorporated by reference) is carried out. Four to thirty female mice, aged approximately 8–10 weeks, are ovariectomized. Administration of a compound of the invention is begun within three

weeks after castration on the experimental group. After one week of administration of a compound of formula 1, the mice are immunized with rat type II collagen. The mice are graded for clinical severity of arthritis, as set out in Holmdahl et al., *Arthritis Rheum.*, 29, 106 (1986), herein incorporated by reference. Sera are collected, and assayed for anti-type II collagen reactive antibodies. At the termination of the experiment, spleen cells are obtained from the mice for determination of T cell activity.

Activity is illustrated by a reduction in titer of anti-collagen type II antibodies determined by conventional ELISA assay. Reduction in T-cell reactivity to type II collagen presented to splenic T-cells by antigen presenting cells is evaluated by quantitation of DNA synthesis by thymidine uptake. Finally, clinical severity of disease is evaluated daily by defining first signs of erythema and swelling of one or more limbs. Clinical assessment is correlated with histologic examination.

Assay 2

Between four and thirty young adult female Sprague-Dawley rats are fed animal chow and water ad libitum. The experimental group receives a compound of formula 1, and all rats receive rat cord generally as described in Arnason et al., *Arch. Neurol.*, 21, 103-108 (1969), incorporated herein by reference. The rats are graded for signs of experimental allergic encephalomyelitis (EAE). Between three and seven weeks after administration of a compound formula 1 began, the rats are sacrificed, their spinal cords removed and examined.

Activity is illustrated by the ability of a compound to inhibit EAE.

Assay 3

Between five and fifty mice (MRL/lpr and NZB) are used. Reduction of anti-DNA antibodies, quantitated by ELISA, as well as changes in survival time and histologic exam of kidneys are evaluated parameters. The mice are dosed with compounds of the invention and are evaluated using the above parameters for disease progression.

Assay 4

Five to fifty women are selected for the clinical study. The women are post-menopausal, i.e., have ceased menstruating for between 6 and 12 months prior to the study's initiation, suffer from an autoimmune disease which exhibits symptoms, but otherwise are in good general health. Because of the idiosyncratic and subjective nature of these disorders, the study has a placebo control group, i.e., the women are divided into two groups, one of which receives a compound of formula 1 as the active agent and the other

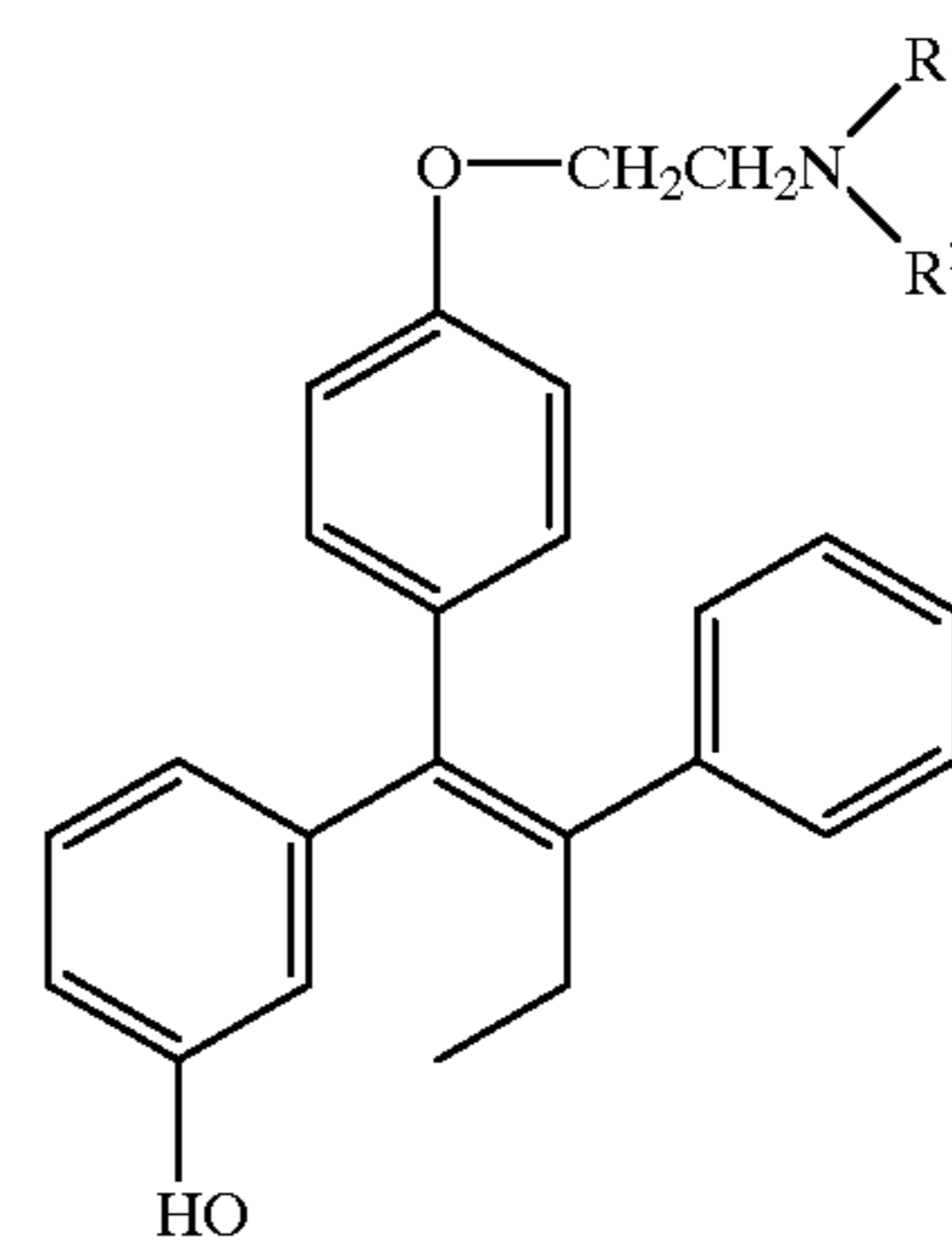
receives a placebo. Women in the test group receive between 50-200 mg of the drug per day by the oral route. They continue this therapy for 3-12 months. Accurate records are kept as to the number and severity of the symptoms in both groups and at the end of the study these results are compared. The results are compared both between members of each group and also the results for each patient are compared to the symptoms reported by each patient before the study began.

Utility of the compounds of formula I is illustrated by the positive impact they have in at least one of the assays described above.

We claim:

1. A method for inhibition of autoimmune diseases comprising administering to a mammal in need of treatment an effective amount of a compound of formula I

(I)



wherein

R¹ and R² may be the same or different provided that, when R¹ and R² are the same, each is a methyl or ethyl group, and, when R¹ and R² are different, one of them is a methyl or ethyl group and the other is hydrogen or a benzyl group; or a pharmaceutically acceptable salt thereof.

2. A method according to claim 1 wherein the compound of formula I is a compound wherein R¹ and R² each are methyl, or a pharmaceutically acceptable salt thereof.

3. A method according to claim 2 wherein said salt thereof is the citrate salt.

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